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Nitrogen alters carbon dynamics during early succession in boreal forest

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ABSTRACT

Boreal forests are an important source of wood products, and fertilizers could be used to improve forest yields, especially in nutrient poor regions of the boreal zone. With climate change, fire frequencies may increase, resulting in a larger fraction of the boreal landscape present in early-successional stages. Since most fertilization studies have focused on mature boreal forests, the response of burned boreal ecosystems to increased nutrient availability is unclear. Therefore, we used a nitrogen (N) fertilization experiment to test how C cycling in a recently-burned boreal ecosystem would respond to increased N availability. We hypothesized that fertilization would increase rates of decomposition, soil respiration, and the activity of extracellular enzymes involved in C cycling, thereby reducing soil C stocks. In line with our hypothesis, litter mass loss increased significantly and activities of cellulose- and chitin-degrading enzymes increased by 45–61% with N addition. We also observed a significant decline in C concentrations in the organic soil horizon from 19.5 ± 0.7% to 13.5 ± 0.6%, and there was a trend toward lower total soil C stocks in the fertilized plots. Contrary to our hypothesis, mean soil respiration over three growing seasons declined by 31% from 78.3 ± 6.5 mg CO_2–C m^{-2} h^{-1} to 54.4 ± 4.1 mg CO_2–C m^{-2} h^{-1}. These changes occurred despite a 2.5-fold increase in aboveground net primary productivity with N, and were accompanied by significant shifts in the structure of the fungal community, which was dominated by Ascomycota. Our results show that the C cycle in early-successional boreal ecosystems is highly responsive to N addition. Fertilization results in an initial loss of soil C followed by depletion of soil C substrates and development of a distinct and active fungal community. Total microbial biomass declines and respiration rates do not keep pace with plant inputs. These patterns suggest that N fertilization could transiently reduce but then increase ecosystem C storage in boreal regions experiencing more frequent fires.

1. Introduction

Nitrogen (N) is known to limit net primary productivity (NPP) (LeBauer and Treseder, 2008), particularly in high-latitude ecosystems with low rates of N fixation and N mineralization (Tamm, 1991; Vitousek and Howarth, 1991). For example, a number of studies have examined the response of boreal forests to N fertilization and found evidence for N limitation of NPP (Mälkönen and Kukkola, 1991; Tamm, 1991; Moilanen et al., 1996). This finding is important because boreal forests are an important source of wood products, and N fertilization can increase forest yields (Mälkönen and Kukkola, 1991). These forests also contain large stocks of C, so the N response of boreal ecosystems could impact global C storage and cycling (Gorham, 1991; McGuire et al., 2009). However, these impacts depend not only on plant responses, but also on soil C responses to N addition.

Contrary to the relatively consistent positive relationship between N and NPP, soil C dynamics respond inconsistently to N (Neff et al., 2002; Waldrop et al., 2004; Knorr et al., 2005). In some systems, NPP responds more strongly to N than decomposition, and ecosystem C storage increases with N addition (Pregitzer et al., 2008). However, other studies have observed that the effect of N addition on C storage depends on ecosystem type and soil organic matter quality (Neff et al., 2002; Waldrop et al., 2004). In a tundra ecosystem near Toolik Lake, Alaska, 20 years of N addition caused

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massive losses of soil C despite increases in plant productivity (Mack et al., 2004). In contrast, soil C storage increased in Finnish boreal forests subject to N fertilization for > 25 years (Mäkipää, 1995). Because these N responses are system-specific, different ecosystems must be studied individually to predict how changes in N availability affect soil C balance.

In the boreal zone, most N fertilization studies have focused on mature forests (Lilleskov et al., 2001; Brenner et al., 2005; Olsson et al., 2005; Allison et al., 2008; Demoling et al., 2008), yet there is considerable landscape heterogeneity in this region (Kasischke, 2000). In particular, fires affect nearly all boreal landscapes and cause sudden and drastic changes in ecosystem properties, such as albedo, C storage, and community structure (Bond-Lamberty et al., 2004; Randerson et al., 2006). Each year, fires consume an average of 11.1 million ha of boreal forest globally (Giglio et al., 2006), and 0.6–1.1% of the Alaskan boreal forest (Lyons et al., 2008). Therefore fires may have implications for biogeochemical processes at the landscape scale.

To explore these implications, we established an N-fertilization experiment across a fire chronosequence in the boreal zone of central Alaska (Treseder et al., 2004, 2007; Mack et al., 2008). Prior work from here indicates that recently-burned boreal systems may respond uniquely to N fertilization due to changes in soil nutrient availability and the structure of plant and microbial communities. Three years after a 1999 wildfire, standing nitrate pools were nearly three times greater in burned relative to unburned soils, although there were no significant differences in ammonium pools (Treseder et al., 2004). During this time, the ecosystem became dominated by mosses, grasses, forbs, and small shrubs interspersed with large areas of bare ground (Mack et al., 2008). Soil microbes were also affected by the fire, with the structure and function of bacterial and ectomycorrhizal fungal communities requiring up to 15 years to recover (Treseder et al., 2004). When the burned ecosystem was fertilized with N, there was a 50 g C m^{-2} decline in soil stocks of glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi (Treseder et al., 2007). These findings indicate that changes in plant and microbial communities resulting from fire may mediate ecosystem C responses to N addition.

Loss of the aboveground plant community means that soil C dynamics will initially control ecosystem C responses to N addition following fire. In boreal ecosystems, fungal communities play a major role in these dynamics by regulating soil C losses through decomposition. Fungi tolerate the acidic pH of many boreal soils (Högberg et al., 2007) and produce extracellular enzymes capable of degrading lignin, cellulose, and organic N, which are dominant components of soil organic matter in boreal ecosystems (Read and Perez-Moreno, 2003; Allison et al., 2009). Therefore, we can gain a more complete understanding of the mechanisms controlling C losses from boreal ecosystems by linking fungal communities with measurements of enzyme activities and decomposition rates.

The objective of this study was to assess the response of soil C cycling and several microbial parameters to N addition in our fire chronosequence site that burned in 1999. We hypothesized that leaf litter decomposition rates would increase because we previously observed that fungal hyphal lengths increased under N addition, suggesting N limitation of microbial activity (Treseder et al., 2007). In conjunction with faster decomposition rates, we expected to find increased soil CO_2 efflux, extracellular enzyme activity, and microbial biomass. Based on laboratory culture studies in which some Basidiomycetes decline in response to N (Zadražil and Brunnert, 1980; Fog, 1988), we predicted that N addition would drive a shift in the fungal community from Basidiomycetes to Ascomycetes. We tested these hypotheses by measuring C pools, CO_2 fluxes, and microbial community characteristics in response to N fertilization.

2. Materials and methods

2.1. Site description and fertilization treatment

The study site is a boreal ecosystem in central Alaska (63°55′ N, 145°44′ W) that experienced catastrophic wildfire in the summer of 1999. A full site description is available in Treseder et al. (2004). All of the dominant canopy trees, mainly black spruce (*Picea mariana*), were killed during the fire and > 50% of the surface organic soil burned. Soils are alluvial silt loams in a region of discontinuous permafrost, although there is no permafrost in the study area (Manies et al., 2004). The vegetation is dominated by grasses (*Festuca altaica*), herbaceous perennials (*Lupinus arcticus*), and small shrubs (*Vaccinium* spp., *Betula glandulosa*, *Ledum groenlandicum*), with aspen and black spruce seedlings beginning to establish (Treseder et al., 2004; Mack et al., 2008). The growing season lasts from bud burst in mid May to leaf senescence in mid September.

A fertilization experiment was established in 2002 as a randomized block design with 4 blocks, each containing 10 m × 10 m control and N addition plots. Nitrogen was applied as NH_4NO_3 in a single dose during June of each year at a rate of 200 kg ha^{-1} yr^{-1} in 2002, and 100 kg ha^{-1} yr^{-1} up to the present. These rates are comparable to additions in other boreal systems (Olsson et al., 2005), and were intended to ensure that no biological processes were limited by N availability. We measured the pH of surface soil (top 5 cm) in a 2:1 (w:w) water:soil slurry in May 2007 and did not find a significant difference with N fertilization (*t*-test, Table 1), probably due to the buffering capacity and relatively high base cation content of these soils (Manies et al., 2004). We assessed the effectiveness of the N treatments using resin bags (Allison et al., 2008). Four anion and 4 cation bags were placed 5 cm below the surface in each plot on May 14, 2006 and retrieved on September 17, 2006; another set of bags was placed in the plots from May 11, 2007 to September 19, 2007. Bags were extracted in 0.1 M HCl/2.0 M NaCl and analyzed for NH_4^+ or NO_3^- concentrations using a modified Berthelot-salicylate method (Weatherburn, 1967) or the vanadium method (Doane and Horwath, 2003), respectively.

2.2. Soil C pools and microbial biomass

We measured soil C pools prior to the initiation of fertilization treatments (2002) and in 2005. Three soil profiles were collected within each plot and combined by genetic horizon (organic or mineral). Organic soils were sampled by digging a small pit, measuring the depth of the layer, and then removing a 25 cm^2 slab of soil. We then collected the top 5 cm of mineral soil with a 2.5 cm diameter corer. All soils were placed on ice for transport to the University of Florida, where coarse debris, roots (> 2 mm diameter), and rocks were removed by hand. Homogenized soils were subsampled for gravimetric water content and bulk C and N, which were determined on an elemental analyzer.

We measured microbial biomass C and N using the chloroform fumigation-direct extraction method on soil cores collected in July 2006 (Brookes et al., 1985; Vance et al., 1987). Five 2 cm diameter × 5 cm depth cores were collected and combined within each plot, then divided into subsamples to determine water content and extractable C and N concentrations with and without fumigation. Biomass pools were calculated assuming a fumigation-extraction efficiency of 0.45 for C (Vance et al., 1987) and 0.54 for N (Brookes et al., 1985).

2.3. Soil respiration

We measured soil respiration rates using flux chambers and an EGM-4 infrared gas analyzer (PP Systems) as reported previously

Table 1
Ecosystem, soil, and microbial characteristics of the control and N-fertilized plots. All soil C data are from 2005, except pre-treatment values. *P*-values are for comparison of control and N-fertilized means.

	Pre-treatment (2002)	Control	Nitrogen	*P*
	Mean ± SE	Mean ± SE	Mean ± SE	
Resin NH_4^+ (μg NH_4^+-N g^{-1} resin d^{-1}) (average 2006–07)		0.146 ± 0.090	9.674 ± 4.228	**<0.001**
Resin NO_3^- (μg NO_3^--N g^{-1} resin d^{-1}) (average 2006–07)		0.00670 ± 0.00378	12.5 ± 3.2	**<0.001**
Soil pH		5.5 ± 0.2	5.9 ± 0.1	0.260
ANPP[a] (g m^{-2} yr^{-1}) (2006)		98 ± 25	255 ± 57	**0.004**
Aboveground Biomass[a] (g m^{-2}) (2006)		171 ± 40	280 ± 52	**0.022**
Organic %C	23.7 ± 1.5	19.5 ± 0.7	13.5 ± 0.6	**0.004**
Mineral %C	7.4 ± 1.1	6.0 ± 0.9	5.1 ± 0.8	0.485
Organic C (g m^{-2})	1548 ± 182	1453 ± 557	545 ± 236	0.177
Mineral C (g m^{-2})	1922 ± 169	1686 ± 59	1678 ± 147	0.960
Total Soil C (g m^{-2})	3470 ± 307	3140 ± 540	2230 ± 250	0.174
Microbial C (μg g^{-1}) (2006)		445 ± 41	171 ± 16	**0.005**
Microbial N (μg g^{-1}) (2006)		41.0 ± 8.5	22.7 ± 9.6	0.175

[a] M. C. Mack, unpublished data.

(Allison et al., 2008). Two 25 cm diameter chamber bases were inserted ~2 cm deep into each plot during May 2006 and left in place for the remainder of the study. To determine soil respiration rates, we placed an opaque lid over each base and measured the accumulation rate of CO_2 in the closed chamber for 5–10 min. Measurements were made at 3–5 time points during each of the 2006–2008 growing seasons. Litter, vascular plants, and mosses were left in the chambers during measurements except on August 19, 2006, when we measured CO_2 fluxes directly before and after removal of aboveground vascular plant biomass. Plant removal had no significant effect on CO_2 fluxes, so we left vegetation intact during future measurements to minimize disturbance and changes in litter inputs. Aboveground plant respiration was unlikely to contribute much CO_2 during May and September measurements since most plant material was senescent at those times.

On September 16, 2006, we also measured the ^{14}C signature of soil respiration in control and fertilized plots using the approach of Czimczik et al. (2006) and Allison et al. (2008). This signature can indicate changes in the source of soil respiration, since soil C fixed 30–50 years ago contains a much higher concentration of ^{14}C due to atmospheric weapons testing at that time. More recently fixed and potentially more labile C sources carry a ^{14}C signature closer to the current atmospheric value, which was ~53‰ at the site in 2006. Thus we would expect microbial respiration from very old or very young C to display lower ^{14}C values than respiration from 30 to 50 year-old C.

2.4. Litter decay rates

Senescent leaves from *F. altaica* (a common grass) and *Vaccinium vitis-idaea* (a common shrub) were air-dried to constant weight and then placed in litter bags (18 cm × 9 cm) constructed of 1 mm fiberglass mesh, and reinforced with 0.2 mm nylon on the lower surface. Each litterbag received 2.0 g total of air-dried litter, either *Festuca*, *Vaccinium* or 1.0 g of each species. The bags were then placed in each of the 8 plots in September 2004, immediately prior to the first snowfall. Four replicates of each litter type were harvested in September 2006. Each litterbag was oven-dried at 60 °C to constant weight. Mass loss was determined as the difference between oven-corrected initial dry weight and final dry weight of the leaves.

2.5. Extracellular enzyme activities

We measured the activities of 4 extracellular enzymes involved in C and N cycling according to established procedures (Allison and Jastrow, 2006; Allison et al., 2008). Three 2 cm diameter × 5 cm depth surface soil cores were collected in each plot and combined by hand at 3–4 time points during the growing seasons of 2006 and 2007. Cores were assayed colorimetrically for the activities of β-glucosidase which releases glucose from cellulose; polyphenol oxidase which depolymerizes lignin and polyphenols; *N*-acetyl-glucosaminidase which hydrolyzes chitin metabolites; and glycine aminopeptidase which degrades proteins and polypeptides. Enzyme substrates were 5 mM *p*-nitrophenyl-β-glucopyranoside, 50 mM pyrogallol/50 mM EDTA, 2 mM *p*-nitrophenyl-β-*N*-acetylglucosaminide, and 5 mM glycine *p*-nitroanilide, respectively, in 50 mM sodium acetate buffer, pH 5.0. For each assay, 150 μl substrate solution was combined with 50 μl soil homogenate. We expressed enzyme activities per gram soil C since soil C concentrations were lower in N-fertilized plots, but we also tested for N effects on enzyme activities per gram dry soil. The soil C concentrations were measured by combustion and elemental analysis on the combined soil sample from each plot at each time point.

2.6. Fungal community structure

To analyze fungal community structure, 10 composite soil cores (2 cm diameter, 5 cm depth) were collected from each plot. Soils were collected on May 29, 2008 and immediately frozen at −20 °C. Total DNA was extracted from a 0.25 g sub-sample of each composite soil sample and triplicate extractions were pooled for each plot using the PowerSoil DNA kit (Mo Bio Laboratories, Inc). Fungal DNA was selectively amplified from soil and litter DNA extractions using the ITS1-F forward primer (Gardes and Bruns, 1993) and the TW13 reverse primer (Taylor and Bruns, 1999). These primers target the ITS and the 28S regions of the fungal genome and produce amplicons of approximately 1200 bp in length. PCR reactions were carried out in 30 μl volumes with 200 mM Tris–HCl PCR buffer, 1.23 mM $MgSO_4$, 0.2 mM of each dNTP, 0.5 μg μl^{-1} BSA, 0.1 μM of each primer, 0.01 U μl^{-1} Platinum *Taq* DNA Polymerase (Invitrogen), and 0.13 μl template DNA ul^{-1} reaction volume. We ran PCR reactions in an iCycler thermocycler (BioRad) with the following program: 5 min initial denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s of annealing at 50 °C, 6 min of elongation at 72 °C, and a final elongation for 10 min at 72 °C. Clone libraries were constructed from PCR products using the pcr 4-Topo TA Cloning Kit for Sequencing (Invitrogen) following the manufacturer's instructions. We picked 50 colonies from each of the 8 plots for a total of 400 clones. Clones were sequenced at Agencourt Bioscience Corporation (Beverly, MA).

Sequences from clone libraries were quality checked and analyzed according to procedures described in Allison et al.

(2007, 2008). We first assembled forward and reverse reads into contiguous sequences where possible using CodonCode Aligner (CodonCode, Inc) and then extracted a ~425 bp region of the 28S rRNA gene to use in sequence alignments. This region started ~625 bp upstream of the reverse primer and included regions of the 28S gene that were variable enough to distinguish taxa but not so variable that the alignment was impossible. Low quality sequences and poorly aligned sequences that matched non-fungal taxa in BLAST searches were removed. Some 28S sequences did not assemble into contigs because of poor quality forward reads, but these were left in the 28S alignment if they originated from fungi. This screening process left 323 sequences for analysis. All sequences are available in GenBank under accession numbers GQ892247–GQ892573.

Sequences were aligned with CLUSTALW (Chenna et al., 2003) and the alignment used for further analyses of fungal community structure. We visually checked the alignment for accuracy, made small adjustments where necessary, and then input the alignment to the Phylip program DNADIST (Felsenstein, 2005) to generate a distance matrix. The distance matrix was input to the program DOTUR (Schloss and Handelsman, 2005) to assign sequences to operational taxonomic units (OTUs) based on sequence similarities of 80–100%. At this point we added 4 ITS sequences that did not form contigs because of poor quality reverse reads, but showed >99% similarity to ITS sequences in contigs that could be assigned to OTUs based on the 28S gene. We calculated the relative abundance of each OTU in each plot and used these abundance distributions to generate a matrix of community distances between all possible pairs of plots in SAS using PROC DISTANCE with the NONMETRIC option (SAS Institute, 2004). This distance matrix was used to create non-metric multidimensional scaling plots of fungal communities and to test for significant effects of N on community structure with multiresponse permutational procedures using the *mrpp* function of the *vegan* package in R (McCune and Grace, 2002; R Development Core Team, 2006). We also used non-parametric Kruskal–Wallis tests to identify significant ($P < 0.05$) N effects on the frequencies of specific OTUs.

2.7. Statistical analyses

Unless otherwise noted, we tested for significant N effects using paired *t*-tests or a mixed-model analysis of variance (ANOVA) with block as a random effect in SAS PROC MIXED. For enzyme activities and CO_2 respiration, we used a repeated-measures ANOVA in SAS PROC MIXED with a compound symmetry covariance structure. We used this design to account for autocorrelation in the data collected from the same plot over time. Resin bag data, microbial biomass, CO_2 respiration, litter mass loss, β-glucosidase activities, and *N*-acetyl-glucosaminidase activities were log-transformed prior to analyses to improve normality. Glycine aminopeptidase and polyphenol oxidase activities were square root-transformed.

3. Results

3.1. Ecosystem C and N pools

We observed large changes in most of the measured C and N parameters in response to fertilization. N addition significantly increased mean soil ammonium and nitrate availability by nearly 2 orders of magnitude according to resin bag analyses (Table 1). Aboveground inputs of plant C also increased, with ANPP more than doubling to 255 ± 57 g m^{-2} y^{-1}, and aboveground biomass increasing significantly by 64% (Table 1). In contrast, belowground C pools declined or remained constant with N addition. There was a significant decline in soil C concentration from 19.5 ± 0.7% to 13.5 ± 0.6% in the surface organic horizon, which helped drive a non-significant trend toward lower C pools in the organic horizon and the total soil (Table 1). Based on pre-treatment soil C pools (Table 1), treatment differences are due to C losses from the N plots rather than C accumulation in the control plots. We did not observe any significant changes in mineral soil C concentration or pool size with fertilization, although C concentrations were slightly lower in the fertilized soils, and significant differences might be difficult to detect due to spatial variability in C pools. Microbial biomass C decreased significantly from 445 ± 41 μg g^{-1} to 171 ± 16 μg g^{-1} under N addition. Microbial biomass N also declined by nearly half, but the trend was not significant.

3.2. Carbon fluxes

Litter mass loss increased significantly with N fertilization, with 3%–9% greater mass loss from litter bags in fertilized plots (Fig. 1). We observed the same effect in each of the litter types individually and in mixed bags with both types.

Despite the increase in litter decomposition, we found that CO_2 fluxes from soil were consistently lower under N fertilization (Fig. 2). On average over the 2006–2008 growing seasons, N addition significantly reduced CO_2 fluxes from 78.3 ± 6.5 mg CO_2–C m^{-2} h^{-1} to 54.4 ± 4.1 mg CO_2–C m^{-2} h^{-1}, with decreases of ~50% observed early in the growing seasons of 2006 and 2007. However, there were no significant differences in ^{14}C signatures of respired CO_2 (69 ± 4‰ in controls versus 76 ± 8‰ in fertilized plots).

3.3. Extracellular enzyme activities

Aside from polyphenol oxidase, extracellular enzyme activities per gram soil C responded strongly to N fertilization. β-glucosidase activities increased significantly and consistently with N addition, from 15.6 ± 0.8 μmol pNP g^{-1} C h^{-1} to 22.6 ± 1.5 μmol pNP g^{-1} C h^{-1} (Fig. 3A). *N*-acetyl-glucosaminidase showed a similar pattern with an increase from 6.5 ± 0.4 μmol pNP g^{-1} C h^{-1} to 10.5 ± 0.8 μmol pNP g^{-1} C h^{-1} (Fig. 3B). In contrast, glycine aminopeptidase activities declined by 61%–2.7 ± 0.5 μmol pNA g^{-1} C h^{-1} with N addition (Fig. 3C). Polyphenol oxidase activity did not change with fertilization (Fig. 3D). When expressed per gram dry soil, glycine aminopeptidase activity declined significantly with N addition ($P < 0.001$), but there were no significant differences in activities of the other enzymes.

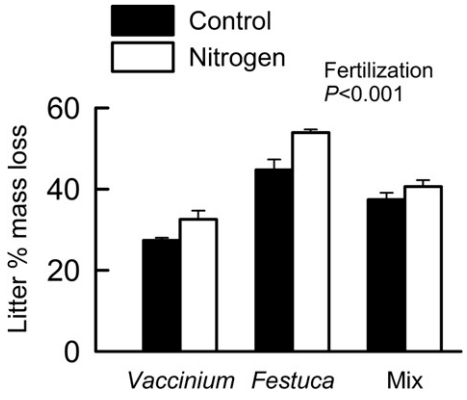

Fig. 1. Mean (±SE) percent mass lost from litter after 2 years of incubation in control and nitrogen-fertilized plots.

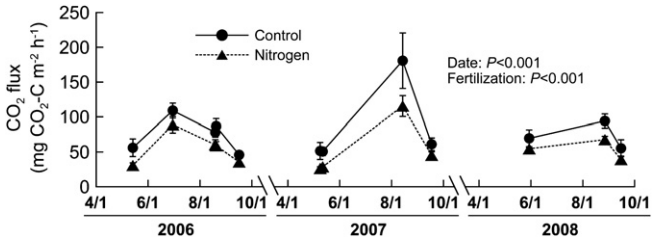

Fig. 2. Mean (±SE) soil CO_2 effluxes in control and nitrogen-fertilized plots during the growing seasons of 2006–08.

3.4. Fungal community structure

Overall we obtained 327 usable fungal DNA sequences from the clone libraries, with 146 from control plots and 181 from N-fertilized plots. We found a total of 110 OTUs at the 99% sequence similarity level with 59 OTUs occurring in the control plots and 71 in the N-fertilized plots (see online Supplementary Appendix for GenBank accession numbers and OTU groupings). When grouped broadly at the 80% sequence similarity level, the majority (55%) of the sequences belonged to an OTU corresponding to the Ascomycota; 26% of the sequences belonged to another OTU corresponding to the Basidiomycota. An OTU containing 9% of the sequences showed the closest correspondence to the Zygomycota. Most of the remaining OTUs could not be reliably classified, but they represented only 15% of the total sequences.

N addition significantly altered fungal community structure at intermediate levels of taxonomic resolution. We did not observe a significant change in community structure at broad levels of taxonomic resolution (i.e. 80% similarity level), although Ascomycota relative abundance increased from 42% to 66% with N addition. MRPP analysis revealed significant changes in community structure at the 95% and 97% sequence similarity levels, with a marginally significant change at the 99% level (Table 2). Consistent with these results, fertilized fungal communities resolved at 95% and 97% sequence similarity were shifted to the left along dimension 1 of the NMS plots (Fig. 4). The weaker response at the 99% level probably occurred because taxa at this level of resolution were more divergent in their N responses, and there were fewer sequences in each OTU, thereby reducing the power to detect significant changes in relative abundance.

Table 2
Changes in fungal community structure with nitrogen addition across levels of taxonomic resolution.

Level of taxonomic resolution	Community shift *P*-value (MRPP)	OTUs with significant changes in relative frequency ($P < 0.05$)	Mean control frequency (%)	Mean nitrogen frequency (%)
80%	0.112			
90%	0.091	90-1 (Sebacinales)	11.6 ± 4.7	0.6 ± 0.6
		90-14 (Ascomycota)	0.0 ± 0.0	2.8 ± 1.0
95%	**0.029**	95-1 (Sebacinales)	10.3 ± 3.2	0.0 ± 0.0
		95-18 (Agaricales)	4.8 ± 3.0	0.0 ± 0.0
		95-20 (Ascomycota)	0.0 ± 0.0	2.2 ± 0.9
97%	**0.029**	97-1 (Sebacinales)	10.3 ± 3.2	0.0 ± 0.0
		97-12 (Ascomycota)[a]	5.5 ± 3.0	27.1 ± 13.0
		97-52 (Ascomycota)	6.2 ± 2.6	0.0 ± 0.0
99%	0.059	99-13 (Ascomycota)[a]	5.5 ± 3.0	27.1 ± 13.0
		99-17 (Ascomycota)	0.0 ± 0.0	5.5 ± 2.3
		99-52 (Sebacinales)	7.5 ± 1.7	0.0 ± 0.0

[a] $P = 0.08$.

Changes in community structure were largely driven by a few groups of fungi. Relative abundance of a group of Basidiomycetes related to the Sebacinales declined significantly under N fertilization (OTUs 90-1, 95-1, 97-1, 99-52), along with a group of Agaricales (95-18, Table 2). Several groups of Ascomycota increased in relative abundance with fertilization, particularly OTU 97-12/99-13, although the increase was only marginally significant and other Ascomycota declined with fertilization (OTU 97-52, Table 2).

4. Discussion

We found strong evidence that N fertilization affects belowground C cycling in our early-successional boreal ecosystem. Several observations support our hypothesis that N addition stimulates SOC decomposition through effects on the microbial community. There was a strong shift toward β-glucosidase and

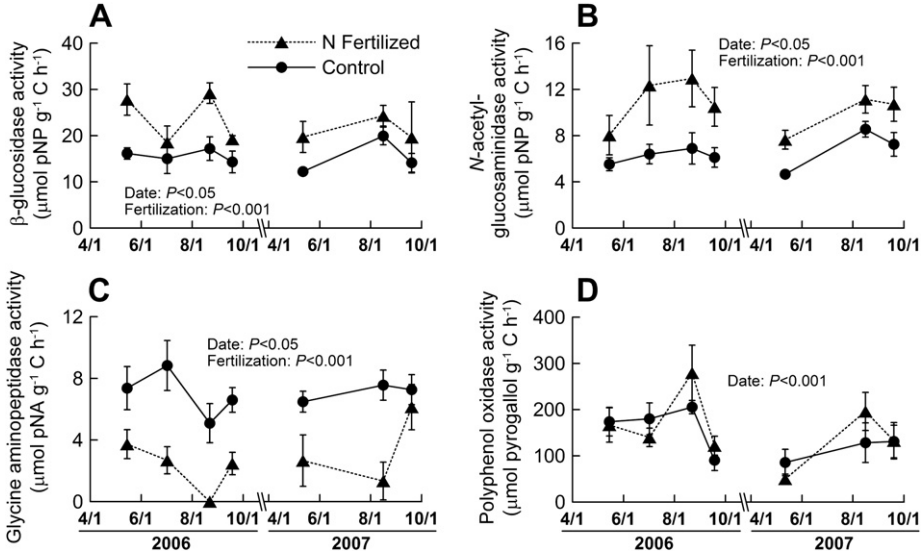

Fig. 3. Mean (±SE) activities of (A) β-glucosidase, (B) *N*-acetyl-glucosaminidase, (C) glycine aminopeptidase, and (D) polyphenol oxidase expressed per unit soil C in control and nitrogen-fertilized plots over the 2006–07 growing seasons.

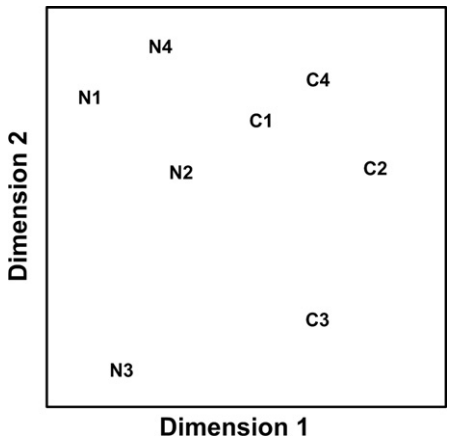

Fig. 4. Non-metric multidimensional scaling plot of fungal communities in control (C) and nitrogen-fertilized (N) plots at the 97% sequence similarity level. Numbers following treatments indicate experimental blocks. Stress = 0.09; $R^2 = 0.94$.

N-acetyl-glucosaminidase activity per unit C in the soil, with much lower glycine aminopeptidase activity. This shift suggests that microbes are allocating resources toward C acquisition rather than N acquisition, which is a common response to N addition in field and microcosm studies (Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005; Allison et al., 2008). When expressed per gram dry soil, β-glucosidase and *N*-acetyl-glucosaminidase activities did not respond to N addition. However, microbial biomass declined significantly (Table 1), suggesting that enzyme production per unit biomass increased for these enzymes but not for glycine aminopeptidase. This pattern is therefore also consistent with re-allocation of resources from N acquisition to C acquisition within the microbial community.

We observed faster litter decomposition rates in N plots, suggesting that enzyme activities were stimulating organic matter turnover. Although we only measured enzymes in soil, higher N availability may have also relieved N limitation of decomposer microbes in the litter layer. A recent meta-analysis found that litter decay rates increased by 17% in response to field N additions of 75–125 kg ha^{-1} yr^{-1} (Knorr et al., 2005), and we have found that low levels of N addition in the laboratory can stimulate enzyme production and the decay of low-N substrates, such as wood and cellulose (Allison et al., 2009). As observed with our soil enzyme data (Fig. 3), litter microbes may have re-allocated resources to C acquisition rather than N acquisition, thereby increasing litter decay rates.

The fungal community was dominated by fungi of the phylum Ascomycota, and their relative abundance increased with fertilization. Ascomycota include fast-growing, weedy species of fungi such as molds, and it is not surprising that they would dominate this early-successional site. They may also be more tolerant of high-N conditions relative to the Basidiomycota (Fog, 1988; Nemergut et al., 2008). We speculate that most Ascomycota groups in this site are N tolerant and probably contributed to increased litter turnover with fertilization. Despite declines in soil microbial biomass, the altered microbial community in fertilized plots was able to sustain relatively high decomposition rates in litter (Table 1, Fig. 1). However, Ascomycetes clearly vary somewhat in their response to N, since OTU 97-52 responded negatively to N addition.

Given that N fertilization increased litter decay rates (Fig. 1) and shifted the plant community toward species with high-quality litter (M. C. Mack, *unpublished data*), we were surprised to find such a sharp decline in soil respiration. This pattern may occur because soil processes influence respiration more than litter decomposition. Reduced respiration from soil could be a legacy of past microbial activity and C substrate depletion under N addition—we observed that C concentrations in the organic horizon declined significantly, and there was a trend toward reduced soil C pools with N fertilization. Low C availability may then have caused the observed reduction in microbial biomass under fertilization and constrained the production of CO_2 from heterotrophic processes. There was little evidence for a change in the fraction of CO_2 derived from autotrophic respiration, since plant allocation to root biomass (Treseder et al., 2007) and ^{14}C signatures of soil respiration (Table 1) did not change with fertilization.

Our results suggest that this recently-burned boreal ecosystem lost a substantial amount of soil C following the initiation of fertilization. Large losses of soil C have also been observed in arctic tundra following N fertilization (Mack et al., 2004), although these losses occurred over a 20 year period. We did not expect to find significant changes in C pool sizes or concentrations after such a short time period at our site. However, the losses we observed are consistent with results from a related laboratory experiment in which we added N to control soils from this site and observed a 40% increase in CO_2 respiration over a 1 year period (Lavoie et al., unpublished manuscript). After 1 year, however, N addition suppressed respiration relative to unmanipulated soils, apparently due to the depletion of labile substrates (M. Lavoie, unpublished data). Therefore we hypothesize that N addition in the field initially caused a large pulse of soil respiration associated with soil C loss, followed by a period of substrate depletion, reduced microbial biomass, and lower soil CO_2 respiration.

Our results can be used to construct a preliminary C budget for the N-fertilized soils. Inputs from aboveground NPP are 255 g m^{-2} yr^{-1}, and inputs from belowground NPP would probably add to this total. We estimate cumulative soil respiration over the 5-month snow-free season to be ~190 g m^{-2} yr^{-1}, although we did not measure winter respiration which could represent 10–20% of annual soil CO_2 efflux (Mast et al., 1998; Welker et al., 2000). Of the total annual respiration, heterotrophic respiration is likely to account for ~50% (Czimczik et al., 2006), which suggests a soil C loss of ~120 g m^{-2} yr^{-1}. Overall, our data suggest that decomposition and heterotrophic respiration may not offset NPP inputs, meaning that N-fertilized soils could be accumulating C. We regard this conclusion as preliminary because of limited temporal sampling of C pools and fluxes, lack of data on leaching and priming effects, and uncertainties in calculating the heterotrophic contribution to soil respiration.

In contrast, unfertilized soils may be losing C or at equilibrium following the burn disturbance. Between 2002 and 2005, we observed a downward trend in all of the soil C measurements in control plots (Table 1). Aboveground NPP in the control plots was only 98 g m^{-2} yr^{-1}, while cumulative soil respiration during the growing season was close to 280 g m^{-2} yr^{-1}. Assuming that soil respiration is 50% autotrophic and that 15% of annual respiration occurs during winter, unfertilized soils would be losing ~165 g m^{-2} yr^{-1} through heterotrophic respiration. With aboveground NPP inputs of 98 g m^{-2} yr^{-1}, belowground NPP would have to exceed 65 g m^{-2} yr^{-1} in order to offset heterotrophic losses. This is unlikely given that Bond-Lamberty et al. (2004) measured belowground productivities of only 2–33 g m^{-2} yr^{-1} in a comparable Canadian boreal ecosystem six years after fire. Although our calculations are too rough to draw definitive conclusions, our data do suggest that the rate of C accumulation is currently higher in N-fertilized relative to unfertilized soils.

Our results indicate that successional stage strongly impacts microbial community and soil C responses to N addition. In a four year study of the mature boreal forest in our fire chronosequence,

we did not observe any change in soil respiration or microbial biomass due to fertilization (Allison et al., 2008). The mature forest is dominated by Basidiomycota rather than Ascomycota, and fungal responses to N addition included shifts in taxonomic composition within the Basidiomycota and a decline in the production of sporocarps belonging to primarily ectomycorrhizal fungi. Thus far, we do not have evidence that N fertilization causes short-term changes in soil C cycling in the mature forest system, in contrast to the early-successional system studied here. Recently-burned ecosystems may be more responsive to N addition because plant and microbial communities are dominated by early-successional taxa that are adapted to growth under high-nutrient conditions (Chapin, 1980; Treseder et al., 2004). Disparate responses to N appear to be typical for boreal ecosystems (Hyvönen et al., 2007), since soil respiration declines and C storage increases in other mature boreal forests following N fertilization (Mäkipää, 1995; Olsson et al., 2005).

5. Conclusions

N fertilization clearly alters C cycling in early-successional boreal ecosystems. These systems may be sensitive to N due to the distinct composition of their plant and microbial communities. In contrast to mature forests, boreal ecosystems at an early stage of succession are dominated by species that respond rapidly to increased N availability in terms of productivity, growth, and C turnover. Although NPP increases, early-successional boreal systems may initially lose C with N fertilization due to increased decomposition rates and changes in the microbial community. However, these C losses may ultimately constrain heterotrophic respiration from soil. It remains to be determined whether reduced soil respiration under N addition will eventually allow C stocks to recover to unfertilized levels, as apparently occurs in some mature boreal forests (Mäkipää, 1995). If not, then increased fire frequencies under global climate change coupled with intensive management practices involving N fertilization could lead to substantial losses of soil C from the boreal zone.

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Appendix. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilbio.2010.03.026.

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